

CLEAN VERSION OF SUBSTITUTE SPECIFICATION

PLANT PATENT APPLICATION



TITLE

5 A NEW BUNASHIMEJI MUSHROOM PLANT NAMED "HOKUTO SHIRO  
ICHIGOUKIN"

LATIN NAME OF THE GENUS AND SPECIES OF PLANT CLAIMED

*Hypsizygus marumoreus*

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The present plant patent application claims priority of Japanese plant variety application No. 14858, filed July 23, 2002, which is hereby incorporated by reference.

15 "Hokuto Shiro Ichigoukin" is a variety of Bunashimeji mushroom plant that was produced by improving the existing varieties to have the following properties: nice white color, no bitter taste, delicious taste with a higher saccharide content, and cultivatable under similar conditions, including cultivation cycle. The scientific name of the variety is *Hypsizygus marumoreus*. The present plant is an edible mushroom.

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BACKGROUND OF THE INVENTION

The present variety "Hokuto Shiro Ichigoukin" was produced using mutant fungal strains of two parent varieties, "Hokuto Hachigoukin" (unpatented) and "Hokuto Jyunigoukin" (unpatented), grown by Hokuto Sangyo Kabushiki Kaisha. Specifically, the 25 + variety was "Hokuto Hachigoukin" and the - variety was "Hokuto Jyunigoukin". "Hokuto Hachigoukin" and "Hokuto Jyunigoukin" were each submitted to UV irradiation, and then the mutated varieties were crossbred to produce the present variety "Hokuto Shiro Ichigoukin".

30 The site of development was on property of Hokuto Sangyo Kabushiki Kaisha Kinoko Sougo Kenkyusho in Nagano, Japan.

In April 2000, the mutated "Hokuto Hachigoukin" was produced, in that a UV-irradiated strain was spawned on a culture medium. In August 2000, the mutated "Hokuto Hachigoukin" was harvested, and monospores were isolated.

In parallel, in May 2000, the mutated “Hokuto Jyunigoukin” was produced, also in that a UV-irradiated strain was spawned on a culture medium. In October 2000, the mutated “Hokuto Jyunigoukin” was harvested, and monospores were isolated.

5 In September and October 2000, primary hyphae were obtained from mutated “Hokuto Hachigoukin” and mutated “Hokuto Jyunigoukin”. From December 2000, mutated “Hokuto Hachigoukin” and mutated “Hokuto Jyunigoukin” were used to obtain crossbred stains. In February 2001, cultivation was started, and harvesting began in May 2001.

10 For cultivation, the cultivated hyphae are typically inoculated in a cultivation medium, like potato dextrose agar (PDA), filled in a sterilized cultivating dish for cultivation and subjected to treatment for growing the mushroom.

Further, in June 2001, a cultivation test was started for secondary selection. In October 2001, an HM222 strain, which appeared to be a superior variety, was selected. In December 2001, a cultivation test was initiated for stabilization.

15 In January 2002, a characteristic test was started. In May 2002, after repeated cultivation tests, stability, reproducibility and homogeneity, matching the objectives of improvements, were confirmed, i.e., the asexually propagated clones of the present plant are identical to the original plant in all distinguishing characteristics. Thus the HM222 strain was named “Hokuto Shiro Ichigoukin”.

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## SUMMARY OF THE INVENTION

The present invention provides a variety, “Hokuto Shiro Ichigoukin”, which is an edible mushroom. When this mushroom is cooked in hot water, the bitterness associated with bunashimeji is mostly gone, thus making it a delectable variety. The present variety 25 can be cultivated anywhere a cultivation facility can be arranged.

In summary, the morphological characteristics of “Hokuto Shiro Ichigoukin” are as follows: at the appropriate harvesting period, the cap is small and round; the color of the cap center and margin is white; the thickness and quality of the cap meat are medium; the number of mottled spots is medium; the mottled spots are distributed in the central area; 30 the clarity of the mottled spots is medium; the color of the gills is yellowish white; the arrangement of the gills is orderly; the width and density of the gills are medium; the length of the stipe is medium; attachment of the stipe to the cap is centric; the shape of the

stipe is moderately thick; the color of the stipe is white; there is no hair on the stipe; and the quality of the stipe meat is medium.

As to the cultivation data for "Hokuto Shiro Ichigoukin", yield is about 118.5 g, the number of effective fruit bodies is 62, and the length of time from spawning to 5 harvesting is 91 days.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a photograph showing a front view of the present plant variety cultivated in vases. The test number designation 00HM069 was used before assignment of the 10 variety name;

Fig. 2 is a photograph showing a front view of the present variety without growth substrate;

Fig. 3 is a photograph showing a perspective view of the present plant variety cultivated in vases;

15 Figs. 4a and 4b are photographs showing a surface and a back, respectively, of a culture of the present plant variety. In these figures, the designation MH025220 is the inventor's company's internal designation for the stock culture of "Hokuto Shiro Ichigoukin";

20 Fig. 5 is a photograph showing a front view of a parent plant variety, "Hokuto Hachigoukin", cultivated in vases;

Figs. 6A and 6B are photographs showing a surface and a back, respectively, of a cultures of parent plant variety "Hokuto Hachigoukin" (the inventor's company's internal designation being MH025019) and of the present invention (designation MH025220), grown in a single dish;

25 Fig. 7 is a photograph showing a front view of a comparison plant variety, "Miyuki M-8171", cultivated in vases; and

Figs. 8A and 8B are photographs showing a top surface and a back surface, respectively, of cultures of plant varieties "Miyuki M-8171" (the grower company's internal designation being MH025066) and of the present invention (internal designation 30 MH025220), grown in a single dish. Figs. 8A and 8B show the state in which fungal hyphae of the present invention and a comparison variety, "Miyuki M-8171" confront each other in a single dish. These fungal hyphae do not commingle, forming a boundary line. This indicates that the two varieties are genetically different from each other. Fig.

8B is a bottom view of the dish shown in Fig. 8A for providing a much better understanding of the boundary line;

Figs. 9A and 9B are a table and a graph, respectively, showing comparative mycelial growth of the present plant variety, as compared with that of parent plant variety 5 “Hokuto Hachigoukin” and comparison variety “Miyuki M-8171”. Fig. 9A represents growth rates of the fungal hyphae cultivated in the range of 22°C to 30°C.

#### DETAILED DESCRIPTION OF THE INVENTION

The characteristics of the present variety will be described in comparison with one 10 of the parent varieties, “Hokuto Hachigoukin”, and with another corresponding variety, “Miyuki M-8171”, which is commercially available (samples were bought at a supermarket in Nagano, Japan).

The cultivation site for this comparison was Hokuto Sangyo Kabushiki Kaisha in Nagano, Japan. The cultivation period was January to June 2002. The cultivation was 15 made according to the conventional methods in a facility with a culture room of approximately 60 m<sup>3</sup> in size and a growth room of approximately 30 m<sup>3</sup> in size. In both the culture and growth rooms, an air-conditioning unit, comprising a cooler and heat exchanger, was installed, and in the growth room, cultivation racks and lights were placed. For characteristic analyses, three cultivation tests were performed using 25 bottles per 20 block. The cultivation method used nutrient propagation, and strains were used as seeds.

In summary, when compared to “Hokuto Hachigoukin”, “Hokuto Shiro Ichigoukin” exhibits the following characteristics: formation of an inhibition zone in dual culture; dense aerial hyphae (“Hachigoukin”: medium density); cross sectional shape of hat is umbonate (“Hachigoukin”: umbonate to flat); white cap center (“Hachigoukin”: 25 yellowish brown); white cap margin (“Hachigoukin”: light grayish brown); medium quantity of mottled spots (“Hachigoukin”: many); central distribution of mottled spots (“Hachigoukin”: all areas except margin); yellowish white colored gills (“Hachigoukin”: white); and medium length stipe (“Hachigoukin”: long).

When compared to the commercial variety, “Hokuto Shiro Ichigoukin” exhibits the 30 following characteristics: formation of an inhibition zone in dual culture; dense aerial hyphae (commercial variety: medium density); clumped pattern for fruit body development (commercial variety: grouped pattern); central distribution of mottled spots (commercial variety: all areas except margin); yellowish white colored gills (commercial

variety: white); a diameter under cap to maximum diameter ratio of 1.59 (commercial variety: 1.6-1.8); and a cap diameter to stripe length ratio of  $\geq 1$  (commercial variety: 1.5-2.0).

In brief the Reference Table below summarizes some of the dimensions of the present invention as compared to the parent varieties, Hokuto Hachigoukin and Hokuto Jyunigoukin, as well as the comparison variety Miyuki M-8171.

Reference Table  
(Dimensions of typical variety of the invention)

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	Hokuto Shiro Ichigoukin	Hokuto Hachigoukin	Miyuki M-8171	Hokuto Jyunigoukin
Cap Diameter (mm) d1	23.2	23.4	24.9	22.5
Stipe Length (mm) L	49.3	56.9	41.5	55.7
Ratio (L/d1)	2.13	2.45	1.68	2.48
Stipe Diameter just beneath Cap (mm)	7.42	5.86	7.80	7.29
Maximum Stipe Diameter (mm)	11.79	9.21	13.27	9.58
Ratio (d3/d2)	1.59	1.58	1.71	1.31
Cap Thickness (mm)	8.0	6.8	7.4	8.2

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The relationship between the parental varieties “Hokuto Hachigoukin” and “Hokuto Jyunigoukin” is explained below: “Hokuto Hachigoukin” has a cap (pileus) with a taupe-color center. “Hokuto Jyunigoukin” is deep gray brown in color. The rim portion of the cap of “Hokuto Hachigoukin” is pale gray brown, and that of “Hokuto Jyunigoukin” is gray brown in color. The cap of “Hokuto Jyunigoukin” is deeper in color on the whole in comparison with “Hokuto Hachigoukin”. Maculae are distributed over the entire cap of “Hokuto Hachigoukin”, but those of “Hokuto Jyunigoukin” are concentrated at the central part of the cap. The stem of “Hokuto Hachigoukin” is white, but that of “Hokuto

Jyunigoukin" is somewhat yellowish in color. Thus, these strains can readily be distinguished in the process of cultivating the strains.

The characteristics of the present plant variety will now be described in details, with relevant comparisons with varieties "Hokuto Hachigoukin" and "Miyuki M-8171".

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#### (1) Dual culture

The test was done according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). With regard to the inhibition zone, the results were shown in Table 1 below (+: inhibition zone, -: no inhibition zone formation).

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Table 1: Inhibition Zone

Similar variety	"Hokuto Shiro Ichigoukin"	"Hokuto Hachigoukin"	"Miyuki M-8171"
"Hokuto Shiro Ichigoukin"	-	+	+

Thus, an inhibition zone was formed between "Hokuto Shiro Ichigoukin" and the comparative varieties "Hokuto Hachigoukin" as well as "Miyuki M-8171", which is the closest variety after "Hokuto Hachigoukin".

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Photographs of the cultures for the present plant variety and for comparative varieties "Hokuto Hachigoukin" and "Miyuki M-8171" are shown on Figs. 4, 6 and 8, respectively.

#### (2) Length of culture from spawning to fruit-body-formation promotion

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The length of culture from spawning to fruit-body-formation promotion was investigated. Again, the test period was January - June 2002. Except for the number of cultivation days, cultivation was done according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). Three lengths of culture were established at 63, 70 and 77 days. The results are summarized in Table 2 below.

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Table 2: Culture Length

Culture length (days)	Growth length (days)	Yield (g/bottle)
63	20.5	128.0
70	20.5	143.4
77	22.0	115.8

Based on the results shown in Table 2, the length of culture was set at 70 days because the yield was the highest for this length.

(3) Optimal budding temperature

To determine the optimal budding temperature, cultivation was done for 70 days according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). Three budding temperatures were established as shown in Table 3 below.

Table 3: Optimal Budding Temperature

Budding temperature (°C)	Budding length (days)
13 ~ 14	6.7
14 ~ 15	6.3
15 ~ 16	6.9

Based on the results shown in Table 3, the minimal temperature was set at 14-15°C because the length of budding was the shortest at this temperature.

(4) Optimal growth temperature

The optimal growth temperature was studied through cultivation for 70 days according to the examination standards established for Shirotamogitake (*Hypsizygus ulmarius*). Three growth temperatures were established as shown in Table 4 below.

Table 4: Growth Temperature

Growth temperature (°C)	Growth length (days)	Yield
13 ~ 14	22.1	115.6
14 ~ 15	21.2	121.1
15 ~ 16	24.9	107.1

Based on the results shown in Table 4, the optimal growth temperature was set at 14-15°C because the yield was the greatest at this temperature.

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(5) Summary of cultivation characteristics

The cultivation characteristics of the present plant variety are shown in Table 5 below.

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Table 5: Summary of Cultivation Characteristics

Characteristics	“Hokuto Shiro Ichigoukin”	“Hokuto Hachigoukin”	“Miyuki M-8171”
Length of culture from spawning to fruit-body-formation promotion (days)	70	70	70
Length of time from fruit-body-formation promotion to fruit body harvesting at optimal temperature (days)	21	21	21
Optimal budding temperature (°C)	14 ~ 15	14 ~ 15	14 ~ 15
Fruit body development pattern	Clumped	Clumped	Group
Yield (g/bottle)	118.5	117.1	105.1
Number of effective fruit bodies	62	100	79
Medium compatibility	Sawdust	Sawdust	Sawdust
Tree selectivity	Mixture	Mixture	Mixture
Cap Size (Diameter) (mm)	23.2	23.4	24.9
Cross sectional shape	Umbonate	Umbonate	Umbonate
Center color	White	Yellowish brown	White
Margin color	White	Light grayish brown	White
Meat thickness (cap depth) (mm)	8.1	6.8	7.4
Meat quality	Medium	Medium	Medium
Mottled spot quantity	Medium	Many	Medium
Mottled spot size	Medium	Medium	Medium
Mottled spot distribution	Central area	All areas except margin	All areas except margin
Mottled spot clarity	Medium	Medium	Medium
Cracking	Absent	Absent	Absent
Gills Color	Yellowish white	White	White
Arrangement	Orderly	Orderly	Wavy and frizzled
Width	Medium	Medium	Medium
Density	Medium	Medium	Medium
Stipe Length (mm)			
Attachment to cap	Centric: eccentric	Centric: eccentric	Centric: eccentric
Shape	Medium thick	Medium thick	Medium thick
Color	White	White	White

Hair	Absent	Absent	Absent
Ratio of maximum diameter to diameter just below mushroom cap	1.59	1.58	1.72
Ratio of length of mushroom stem to diameter just below mushroom cap	2.1	2.45	1.68
Meat quality	Medium	Medium	Medium

The color designations are based on the Japan Color Standard for Horticultural Plant (JHS Color Chart). Other evaluations like the words "medium" are based on the standards stipulated by the Agriculture Forestry and Fisheries Ministry of Japan, under which strains  
5 may be evaluated with the eye.

#### (6) Cultivation characteristic data

10 Detailed cultivation characteristics of the present plant variety are shown in Table 6 below. The color designations are based on the Japan Color Standard for Horticultural Plant (JHS Color Chart). The code numbers mentioned in the column "Code of the similar variety" below are based on color codes stipulated by JIS (Japanese Industrial Standard). Other evaluations like the words "medium" are based on the standards stipulated by the  
15 Agriculture Forestry and Fisheries Ministry of Japan, under which strains may be evaluated with the eye.

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Table 6: Detailed Cultivation Characteristics

Characteristics	Characteristic code of filed variety										Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09	Hokuto Hachigoukin		Miyuki M-817	
Cultivation characteristics													
Length of culture from spawning to fruit-body-formation promotion (days)	40以下	41~50	51~60	61~70	71~80	81~90	91~100	101~110	111以上	70days	04	04	
Length of time from fruit-body-formation promotion to fruit body harvesting at optimal temperature (days)			19以下		20~25	26以上				21days	05	05	
Optimal budding temperature (C)	10 and under	11~14	15~18	19~22	23~26	27 and over				14~15	03, 04	03, 04	
Optimal developed temperature (C)	8 and under	9~11	12~14	15~17	18~20	21 and over				14~15	04, 05	04, 05	
Optimal intensity													
Budding period			Early		Intermediate		Late						
Fruiting habit	Grouped	Scattered	Clumped						other	Clumped	03	01	
Yield (g/bottle)	80以下	81~100		101~120		121以上				118.5	06	06	
Number of effective fruit bodies	20以下	21~25	26~30	31~35	36~40	41以上				62.0	07	07	
Medium compatibility	Log wood	Sawdust							other	Sawdust	02	02	
Tree selectivity	Broad leaved	Coniferous	mixtu						other	Mixture	03	03	

Characteristics	Characteristic code of filed variety										Note (Data)	Code of the similar variety		
	01	02	03	04	05	06	07	08	09			Hokuto	Hachigoukin	Miyuki M-817
Morphology														
Cap														
Size			small		medium		large			23.2(mm)		03	03	
Cross sectional shape	Conv ex	Umbonate	expanded	infundibuliform					other	Umbonate		02, 03	02	
Center color	W	GY	LG B	YB	GB	DY B	DGY B	DG B	other	White		04 9YR1916	01 No4-01	
Margin color	W	G-Y	LG B	YB	GB	DY B	DGY B	DG B	other	White		03 9YR1915	01 No4-01	
Thickness			Thin	medium		Thick				8.1(mm)		05	05	
Hardness			Soft	Medium		Hard				Medium		05	05	
Mottled spot quantity			Few	Medium		Many				中		07	05	
Mottled spot size			small	medium		large				中		05	05	
Mottled spot distribution	All areas except margin	Central area						other	Central area			02	02	
Mottled spot clarity			unclear	medium		Clear				Medium		05	05	
Cracking	Absent							Present	Absent			01	01	
Gills														
Color	W	YW	LO	GY					other	Yellowish white No. 4-2501		01	01	
Arrangement	Orderly	Sinuate							other	Orderly		01	02	

Characteristics	Characteristic code of filed variety									Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09		Hokuto Hachigoukin	Miyuki M-817
Width			Narrow Spac ed		Medium		Wide Dense			Medium	05	05
Density					Medium					Medium	05	05
Stipe												
Length			Short		Medium		Long			49.3(mm)	07	03
Attachment to cap	Centric	Eccentric							Other	Centric 7: eccentric 3	01	01
Shape	Thin Long	Thin Short	Thick Long	Thick Short	Medium	Thick			Other	Medium thick	05	05
Color	White								Color ed	White	01	01
Hair	Absent								Present	Absent	01	01
Diameter under cap to maximum diameter ratio			1.5 and under		1.6 ~ 1.8		1.9 and over			1.59	03	05
Cap diameter to stipe length ratio			1.4 and under		1.5 ~ 2.0		2.1 and over			2.1	07	05
Hardness	Soft				Medium		Hard			Medium	05	05
Others characteristics												

Characteristics	Characteristic code of filed variety									Note (Data)	Code of the similar variety	
	01	02	03	04	05	06	07	08	09		Hokuto Hachigoukin	Miyuki M-817
Genetic Dual culture												
Inhibition zone	—								+	01	09	09
Physiology												
Mycelial growth												
Effect of temperature												
The optimum(°C)	22	23	24	25	26	27	28	29	30	24°C	04	03
Growth rate												
5°C										4.0mm/day	4.9	6.4
10°C										9.3mm/day	12.2	13.7
15°C										19.9mm/day	18.7	20.5
20°C										25.8mm/day	24.3	26.3
25°C										28.6mm/day	30.6	31.2
30°C										11.6mm/day	15.5	16.9
35°C										0.8mm/day	2.6	1.2
Mycelial density			Thin		medium		Thick			Thick	05	05
Development of aerial hyphae			Few		medium		Many			Medium	03	05

In summary, the “Hokuto Shiro Ichigoukin” is white in color and can be discriminated in variety by the boundary line seen when being cultivated in a dish with another different variety. By comparison , wild “Bunashimeji Shirotamogitake” (*Hypsizygus ulmarius*) is not white. “Hokuto Shiro Ichigoukin” tastes delicious when boiled while other similar varieties taste bad.